Drug Resistance

A SURVEY OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS

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IN an earlier symposium on the resistance of bacteria to the penicillins, Barber (1962) emphasized the difficulties of obtaining an estimate of the incidence of methicillin resistance in *Staphylococcus aureus*. To yield reliable information, the population under investigation must be large and diverse, and must be sampled in a uniform way; and the degree of resistance to be recorded must be defined precisely. In the survey we began in 1960 we have tried to fulfil these criteria.

During the last three and a half years we have carried out tests for methicillin resistance on 43,867 of the 60,053 cultures of *Staph. aureus* we received for typing. The way in which they were selected has already been described (Jevons, Coe and Parker, 1963). Most of them came from hospitals in south-eastern England and were isolated from hospital patients and staff, but we excluded all cultures sent to us because they had been found to be methicillin resistant.

We used a screening test in which filter-paper discs containing 10 μg. methicillin were placed on plates inoculated from 6-hour broth cultures. Cultures naturally resistant to methicillin appear to consist of a minority of individuals with a high resistance and a majority whose resistance is only slightly increased (Knox, 1961; Knox and Smith, 1961; Sutherland and Rolinson, 1964). The appearance of a disc-sensitivity test on a resistant culture depends, therefore, on the proportion of highly resistant individuals it contains. Some cultures grow continuously up to the disc, but others show no more than a slight narrowing of the zone of inhibition with a few discrete colonies nearer to the disc (Fig. 1).

Cultures which were apparently resistant to methicillin when tested with discs were also tested by a tube-dilution method, and were finally regarded as resistant only if the minimum inhibitory concentration (M.I.C.) was 12.5 μg. methicillin/ml. or more after 18 hours incubation. (For full details see Jevons *et al.*, 1963).

Incidence

In the three months October to December, 1960 we examined 5,440 cultures of *Staph. aureus* and found three that were methicillin resistant, all from one hospital (Jevons, 1961). One of the resistant organisms came from a patient from whose nose and skin *Staph. aureus* had previously been isolated in July, 1960. Fortunately the earlier cultures had been kept. They were found also to be methicillin resistant, though the M.I.C. for methicillin was lower than that of the organisms isolated later. Methicillin was not used in this hospital before October, 1960. Several other accounts have been published of the appearance of methicillin-resistant *Staph. aureus* in places where methicillin had not been used (for example, Cetin and Ang, 1962; Borowski, Kamiénska and Rutecka, 1964). It is likely, therefore, that the origin of naturally methicillin-resistant strains of *Staph. aureus* is unconnected with the use of methicillin.

Since our first survey (Jevons, 1961), we examined several thousand cultures in each subsequent quarter-year. During 1961 and 1962 there was a slow increase in the proportion of resistant cultures, but their incidence appears to have changed little since the middle of 1962 (Table I). Our figures for 1961 and 1962 were, however, rather over-weighted by the inclusion of a large number of resistant cultures from Queen Mary's Hospital for Children, Carshalton, where an exhaustive search for resistant organisms was being made. If the Carshalton cultures are excluded we have a truer estimate of changes in the incidence of resistance. Fig. 2 shows that, up to the middle of 1962, the percentage of resistant cultures (excluding Carshalton) fluctuated between 0.06 and 0.29. In the third quarter of that year it rose to 0.61 and has remained at about that level since.

Table II shows the total number of cultures which came from each of the laboratories submitting methicillin-resistant staphylococci, and the number from each source that were resist-
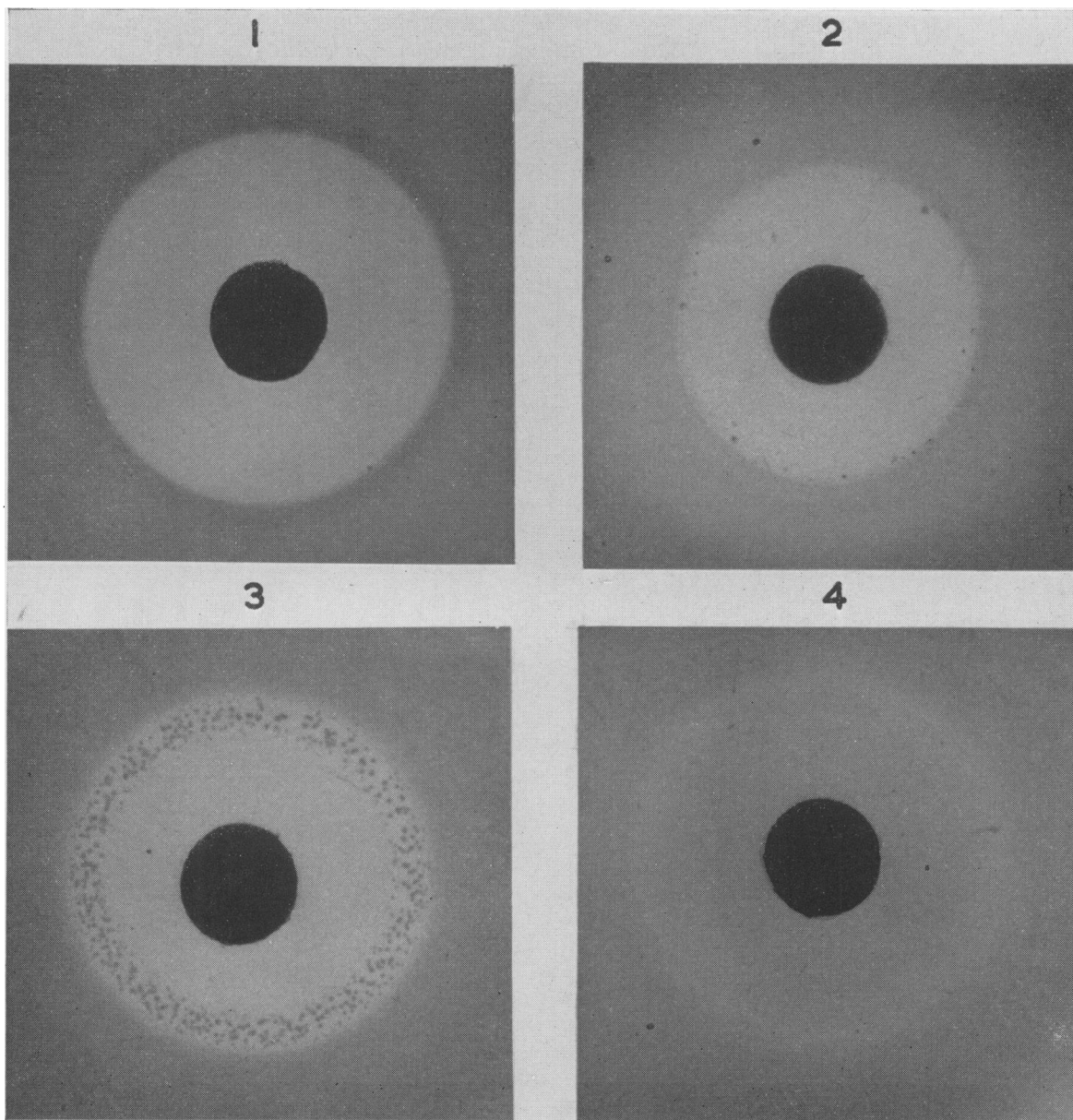
FIG. 1.—*Disc sensitivity tests with methicillin.* Each disc contains 10 μg. methicillin. (1) methicillin-sensitive culture, (2), (3) and (4) resistant cultures (see text).

ant in each three-month period. Only one resistant culture from each infected person was included. Resistant organisms were received from 35 laboratories. Their sources included 31 different British hospitals, three foreign hospitals, and the Food Hygiene Laboratory at Colindale. A total of 198 hospitals in Great Britain sent us cultures, and one or more methicillin-resistant culture was obtained from

16% of them. The number of resistant cultures from one hospital was seldom large, and was in most cases a very small proportion of all the cultures sent. Indeed, there was a close correlation between the total number of cultures submitted and the chance that they would include a resistant organism. In the first two years of our survey (Jevons *et al.*, 1963), no resistant cultures were ob-

TABLE I

Isolations of Methicillin-Resisant *Staphylococcus aureus*

| Year	Quarter	Cultures examined	Resistant*	Laboratories contributing resistant cultures	Cumulative total of laboratories contributing resistant cultures
1960	4	5440	3 (0.06)	1	1
1961	1	4135	8 (0.19)	4	4
	2	3008	15 (0.49)	3	6
	3	2101	13 (0.61)	6	10
	4	3708	11 (0.30)	5	13
1962	1	3179	14 (0.44)	7	18
	2	3454	18 (0.52)	3	19
	3	2454	20 (0.81)	8	23
	4	2779	23 (0.83)	8	24
1963	1	2613	20 (0.77)	10	26
	2	3396	23 (0.68)	7	27
	3	2715	16 (0.59)	6	30
	4	2386	15 (0.63)	10	33
1964	1	2499	14 (0.56)	6	35
Total		43,867	213 ..	..	..

*percentage in parenthesis.
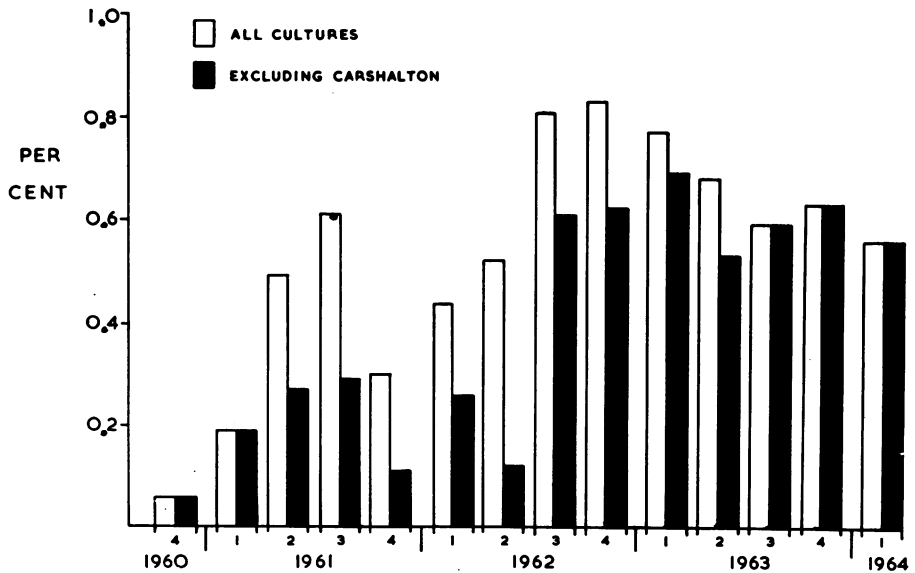
FIG. 2.—Percentage of *Staph. aureus* cultures resistant to methicillin in each quarter from October, 1960 to March, 1964.

TABLE II

Source of Resistant Cultures of *Staph. aureus*

Laboratory	Cultures submitted	1960 Quarter 4	1961 1	2	3	4	1962 1	2	3	4	1963 1	2	3	4	1964 1	Total	Distinct strains per laboratory
1	526	3	4	6	2		1				2					18	1
2	745		2					3								5	2
3	100		1													1	1
4	154		1													1	1
5	283			7	7	7	6	14	5	6	2	5				59	1
6	111		2													2	1
7	3996				1						2	1				4	1
8	77				1											1	1
9	98				1	1			1	5	1	1		1	1	12	2
10	1024				1											1	1
11	207					1								1		2	1
12	223					1										1	1
13	689					1										1	1
14	34						1	6								7	1
15	1923						1				5					6	2
16	25						3									3	1
17	1383						1		1	1	3	1	1	3		11	1
18	657						1				1					2	2
19	305							1		1		1	1	1		5	2
20	242							1					10	4		15	1
21	35							4	5							9	1
22	67							1	1						1	3	2
23	648							1	2		1	3				7	2
24	43								2							2	1
25	1788										1		1			2	1
26	9										2					2	1
27	488											11				11	1
28	208												2			2	1
29	200												1			1	1
30	88												1	2		3	1
31	326													1	1	2	2
32	99													1	2	3	1
33	338													1		1	1
34	10664														1	1	1
35	116															7	1

tained from any hospital sending less than ten cultures, but 10%, of hospitals sending between 10 and 99 cultures, 24% of those sending 100 to 999 cultures and half of those sending 1,000 or more cultures included a resistant organism.

There were a few hospitals, however, in which a single resistant strain of *Staph. aureus* was endemic for long periods (e.g., Nos. 1, 5, 9, 17 and 19). No. 1 was the hospital in which a resistant organism was first identified in July 1960, and occasional isolations of the same strain were made until early 1963. Stewart and Holt (1963) have given a full account of the prevalence of a resistant strain in Queen Mary's Hospital, Carshalton (No. 5); 59 of the cultures in our series came from this source. Despite the fact that a careful search was made for infected patients in this hospital, the resistant organism was only isolated from about 2% of the patients admitted during one year.

Sepsis

Table III shows the situations in which methicillin-resistant cultures of *Staph. aureus* were isolated. Only one from each patient was included; when the organism was isolated both from a lesion and from a carrier site, the lesion was given as the source. Nearly half of the 213 isolations (48%) were from lesions thought to be staphylococcal. They included three cases of septicæmia, two of meningitis, and eight lung infections confirmed *post mortem*. A quarter were from infected wounds.

Characters

All the resistant cultures belonged to phage

TABLE III

Site of Isolation of 213 Methicillin-Resistant Cultures

Lesions		
Septicaemia	3	
Meningitis	2	
Lung infections	8	
(post-mortem)		
Abscess	6	
Wound infection	51	
Superficial skin lesion	13	
Urinary infection	12	
Other or unspecified	7	
Total	102	(48%)
Sputum	34	
Carriers	65	
Objects	5	
Not known	7	
	213	

TABLE IV

Phage-Typing Patterns of Methicillin-Resistant *Staph. aureus* Cultures

Phage-typing patterns	Strains	Foci of infection†
7/47/53/54/75/77/+	101	20
75/77/+	68	6
6/53/54/75/77/+	10	4
Untypable		
(+ group III inhibition)*	25	5
83A*	4	3
77	1	1
42E/54/77/81	1	1
75	1	1
75	1	1
7	1	1

†Number of hospitals from which each strain was obtained.
*Closely related.

In italic: reactions at 1,000 times the routine test dilution

group III (Table IV) and nearly all of them (98%) had one of five phage-typing patterns. Until early in 1963 the commonest pattern was 75/77/+, but most of these isolations were made in Hospital 5. In the present series, cultures with the pattern 7/47/53/54/75/77/+ were the most numerous, and were obtained from the greatest number of different foci.

The organisms described in our earlier paper (Jevons *et al.*, 1963) as "29 (+ group III inhibitions)" have been listed in Table IV as "Untypable (+ group III inhibitions)", because they are now known to be examples of the "new" untypable staphylococcus believed to have arisen by the lysogenization of type 83A strains (see Jevons and Parker, 1964). Organisms of this sort were first observed in Britain in 1960 and have since become very widespread (Temple and Blackburn, 1963; Jacobs, Willis, Ludlam and Goodburn, 1963; Turner, 1963; Jevons and Parker, 1964). We examined large numbers of them in 1961 and 1962, but it was not until 1963 that methicillin-resistant organisms were found among them in this country. They have since become endemic in several hospitals. Similar organisms isolated in 1962 in Switzerland and South Africa were methicillin-resistant (Jevons *et al.*, 1963).

We have recently seen two undoubted examples of naturally occurring methicillin-resistant organisms which do not belong to phage group III. One was a completely untypable organism from Denmark, sent to us by Dr. K. Riewerts Eriksen, and the other was a phage group I culture (lysed by phage 29 at routine test dilution) from a British hospital. It has repeatedly been observed that when staphylococci resistant to a particular antibiotic first become prevalent they have a restricted range of phage-typing patterns, but that in time fresh strains with new patterns appear. It will not be surprising, therefore, if methicillin-resistant members of other phage groups become common in due course.

The other cultural characters of methicillin-resistant strains of *Staph. aureus* are those commonly seen in hospital staphylococci. We have examined in detail 36 cultures, each representing an independent focus of infection. All were resistant to penicillin, streptomycin and tetracycline; six were resistant to chloramphenicol and two to erythromycin. All 36 were mercury resistant. We reported earlier (Jevons *et al.*, 1963) that none of the first 16 independent cultures we tested was lipolytic for Tween 80, but we have since seen several Tween-positive cultures.

Ayliffe and Barber (1963) showed that, like other strains of *Staph. aureus* resistant to several antibiotics, methicillin-resistant organisms usually produced large quantities of penicillinase. This was confirmed when the production of penicillinase was measured under standard conditions (Richmond, Parker, Jevons and John, 1964). Figure 3, which is constructed from figures given by Richmond and his colleagues (1964), shows that 20 of 23 methicillin-resistant cultures of *Staph. aureus* gave high yields of penicillinase (factors of 0.9 or more) but not higher that those given by many other multiple-resistant but methicillin-sensitive cultures.
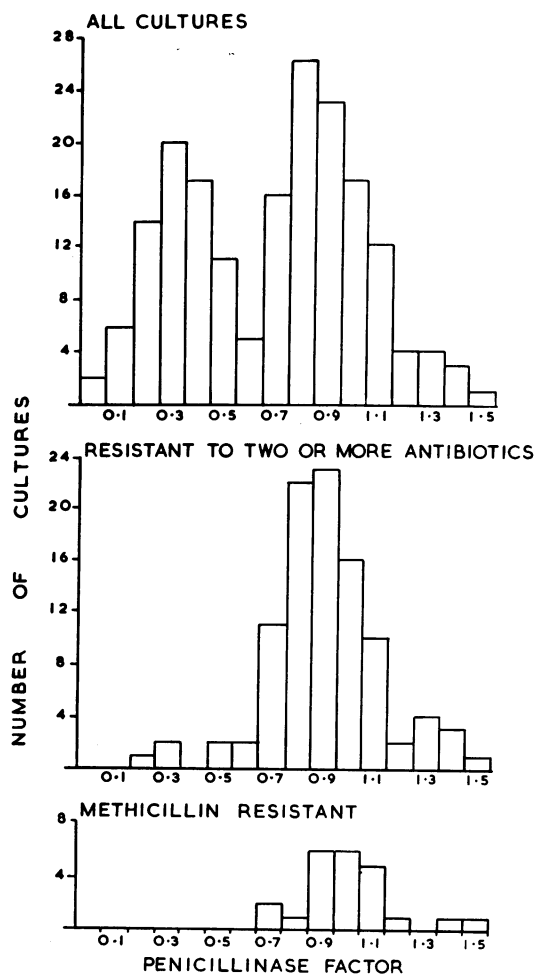
FIG. 3.—Quantity of penicillinase synthesized by cultures of *Staph. aureus* (from Richmond, Parker, Jevons and John, 1964).
Top: whole series of 181 cultures
Middle: 82 cultures resistant to two or more antibiotics
Bottom: 23 methicillin-resistant cultures.
A factor of 1.0 is equivalent to a penicillinase production of 330 enzyme units mg. bacteria.

The significance of methicillin resistance

There is little evidence that the use of methicillin has so far played much part in the spread of resistant strains. As far as we could ascertain, the hospitals in which resistant strains were widespread were not ones in which methicillin had been used excessively for therapeutic or prophylactic purposes. The antibiotic appears to have been used with restraint in most hospitals. Enquiries by a Public Health Laboratory Service committee in 1961-62 indicated that less than 1% of the patients in medical wards in ten provincial hospitals had received methicillin. The proportion is probably higher than this in certain special units, but methicillin appears seldom to have been used as freely as other antibiotics to which *Staph. aureus* has frequently been found to be resistant. There are several reports of the prophylactic use of methicillin as a spray in single hospital wards (Elek and Fleming, 1960; Ulstrup and Odegaard, 1961; Stewart, Holt and White, 1961; Goldfarb and James, 1963). In none of them was a methicillin-resistant organism isolated from any of the occupants of the ward. There is little information about the frequency with which spraying has been used as a routine procedure elsewhere, and none at all of its consequences in wards in which a resistant organism is present.

Borowski and his colleagues (1964) reported recently that over 50% of the mothers and babies in a hospital in which methicillin had never been used became colonized with a methicillin-resistant strain of *Staph. aureus*. This observation raises the question whether insusceptibility to methicillin might confer some other advantage on a staphylococcus. Hitherto, the accepted view has been that, in the absence of methicillin, resistance is an irrelevant or "accidental" property of strains which possess the other characters of hospital staphylococci, to which their spread in the hospital population may be attributed, for example, the active production of penicillinase and resistance to other common antibiotics.

It is well known that strains of *Staph. aureus* that have been rendered artificially resistant to methicillin *in vitro* also show an increased tolerance to benzyl penicillin (Knox and Smith, 1961; Barber, 1961). It was inferred that naturally occurring methicillin-resistant strains had the same property, but the fact that they all produced large amounts of penicillinase made this difficult to prove. We found it possible, however, to select penicillinase-negative variants from methicillin-resistant cultures by the method of Novick (1963) and investigated the action of benzyl penicillin on them. As expected, it resembled the action of methicillin on naturally methicillin-resistant staphylococci. The population in these cultures consisted of a minority of organisms with a high resistance to benzyl penicillin, and a majority with only a slightly increased resistance. In a disc sensitivity test with one unit benzyl penicillin the zone of inhibition was

TABLE V

Sensitivity to Benzyl Penicillin of a Penicillinase-Negative Variant of a Methicillin-Resistant Culture of *Staph. aureus* (No. 5562) Measured by Tube-Test.

Culture	Benzyl Penicillin units										
	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.095	0.05	0.02	0.01
5562: penicillinase— negative variant	— (±)	— (+)	— (+)	— (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
Oxford Staphylococcus	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (±)	± (+)	+ (+)

The Oxford Staphylococcus was tested at the same time. Inoculum: 1 drop of a 6-hr. culture. At 24 hr. + = turbidity ± = slight turbidity — = no turbidity. In parenthesis: results at 48 hr.

narrow and hazy at the edge, and with larger amounts of benzyl penicillin (three or six units) a number of discrete colonies could be seen within the zone.

The behaviour of the same cultures in a tube-sensitivity test with benzyl penicillin also resembled that seen with methicillin-resistant organisms in the presence of methicillin (Rolinson, 1961). Table V shows the results of an experiment in which 0.02 ml. of a six-hour broth culture was added to tubes each containing a dilution of antibiotic in one ml. broth. The Oxford Staphylococcus had a M.I.C. of 0.05 units/ml. and the penicillinase-negative variant of 5562 had a M.I.C. of 1.56 units/ml. at 18 hours. After 48 hours, however, the methicillin-resistant organism had grown in 12.5 units of benzyl penicillin/ml. If the penicillin-broth mixture was incubated at 37°C. for 18 hours before inoculation, the M.I.C. of the Oxford Staphylococcus was increased only two-fold. The M.I.C. at 18 hours for benzyl penicillin of penicillinase-negative variants of seven other methicillin-resistant organisms were between 0.09 and 3.12 units/ ml. (i.e., 2 to 64 times that of the Oxford Staphylococcus), but all showed growth in 6.25 units/ml. or more after 48 hours.

It is possible, therefore, that it is the natural insusceptibility of these organisms to penicillins in general, rather than their resistance to methicillin, which has so far contributed to their limited success as "hospital staphylococci". The few individuals highly insusceptible to benzyl penicillin might be able to survive and to multiply slowly in the presence of the antibiotic (Sutherland and Rolinson, 1964) in environments in which the number of staphylococci was not sufficient to produce an adequate concentration of penicillinase. The fact that insusceptibility to penicillins has so far been found only in strains which possess several other characters common to staphylococci endemic in hospitals suggests, however, that it may not be of decisive importance for survival and colonization. An alternative but less attractive hypothesis is that insusceptibility to penicillins is closely linked genetically to some other significant character.

It is not surprising that methicillin-resistant staphylococci can, upon occasion, spread widely in a hospital in which large amounts of penicillins are being used, but the frequent persistence of a strain for long periods at a very low level of endemicity has to be explained. Many of the hospitals regularly sending us large numbers of cultures included less than one resistant culture a month, and it is at first sight difficult to see how the chain of infection was maintained. Part of the explanation is given by the investigations of Stewart and Holt (1963). At Carshalton, most of those

who were infected became nasal carriers but suffered from no staphylococcal illness. The first severe infection was not observed until 36 other persons had acquired the organism. In Gdańsk (Borowski *et al.,* 1964), over 100 persons carried the organism in the nose but there was no sepsis. It seems, therefore, that in many foci of infection the nasal carriers greatly outnumber the cases of clinical infection, and unless large numbers of cultures from nasal swabs are tested for methicillin resistance the connection between successive clinical infections will be missed.

It is probable also that the disc method of screening may give us a rather low estimate of the incidence of methicillin resistance. It depends on the presence in the inoculum of individual staphylococci with a sufficiently high resistance to form discrete colonies within the zone of inhibition. As we saw in Fig. 1, the number of such individuals varies greatly. On several occasions we have seen organisms from hospitals in which a methicillin-resistant strain was endemic which had a phage-typing pattern identical with that of the resistant organism, but which appeared to be sensitive in the disc test. There have also been occasions on which the disc test suggested that an organism was resistant but the M.I.C. was less than 12.5 μg./ml.

We therefore selected a group of strains with identical phage-typing patterns from one hospital, including three that were resistant and five that were sensitive to the disc test. All were resistant to penicillin and at least one other unrelated antibiotic. Two of the three apparently methicillin-resistant cultures had a M.I.C. of only 6.25 μg. methicillin/ml., and one of the five cultures sensitive in the disc test had a M.I.C. of 25 μg./ml. Penicillinase-negative variants were obtained from the five "sensitive" cultures and tested for sensitivity to benzyl penicillin. Four of them showed evidence of inherent resistance to benzyl penicillin, and had M.I.C.s of 0.39, 0.39, 1.56 and 3.12 units/ml. respectively at 18 hours. It seems, therefore, that our definition of a methicillin-resistant *Staph. aureus* as an organism with a M.I.C. of at least 12.5 μg. methicillin/ml. is too strict, and that the disc test is not sensitive enough to detect all the cultures showing some natural insusceptibility to penicillins. Cultures from the same focus of infection differ considerably in the proportion of more resistant individuals they contain, and this makes it difficult to define the true extent of the spread of a resistant strain.

Conclusion

The results of our survey raise a number of difficult questions about the significance of methicillin resistance in *Staph. aureus,* and it is unlikely that further information collected in the same way will help much in solving them. What we now need is a series of more detailed studies in particular hospitals, in which the acquisition of resistant organisms by patients is related to the therapeutic use of various penicillins, and to the presence of antibiotic in the environment. A more suitable screening test for use in such surveys would be to inoculate heavily a single tube of broth containing perhaps 12.5 μg. methicillin/ml. and to incubate for 48 hours. (See also Sutherland and Rolinson, 1964).

Summary

Between October, 1960 and March, 1964 nearly 45,000 cultures of *Staph. aureus* were examined for methicillin resistance by a screening test with paper discs containing the antibiotic.

Naturally resistant organisms almost certainly existed before methicillin was synthesized in the laboratory, but have probably increased in frequency in the last few years.

Their distribution is rather patchy. An occasional methicillin-resistant *Staph. aureus* was found in a high proportion of those hospitals from which many cultures were examined. In a few hospitals a single resistant strain became endemic and persisted for months or years. There was little evidence that this was directly related to the use of methicillin.

Nearly all the resistant cultures belonged to phage group III, and had one of a small number of phage-typing patterns. They produced large quantities of penicillinase, were resistant to mercury salts, and resistant also to one or more antibiotics unrelated to penicillin.

Methicillin-resistant staphylococci occasionally spread in hospitals in which methicillin has not been used. This directed our attention to their natural insusceptibility to benzyl penicillin. Penicillinase-negative variants of methicillin-resistant cultures consisted of a minority of individuals with a rather high resistance to benzyl penicillin, and a majority with only a slightly increased resistance. This suggested a possible mechanism for survival in the presence of penicillin under conditions in which the organisms were too few to produce an adequate concentration of penicillinase.

A very low level of endemicity of methicillin-resistant strains was observed in some hospitals. This was explained in part by the frequency of undetected symptomless infections, but there was also evidence that neither the disc sensitivity test, nor the tube sensitivity test as it is usually performed, was sufficiently sensitive to detect all resistant organisms.

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METHICILLIN-RESISTANT STAPHYLOCOCCI AND HOSPITAL INFECTION

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METHICILLIN-RESISTANT strains of *Staph. aureus* have now been isolated from patients in hospitals in Britain (Jevons, 1961; Barber, 1961; Knox and Smith, 1961), France (Chabbert and Baudens, 1962), Turkey (Cetin and Ang, 1962), Denmark (Eriksen and Erichsen, 1963) and Poland (Borowski, Kamienska and Rutecka, 1964). The resistance appears to be naturally occurring since in most cases the strains have been isolated from patients who have never had any of the penicillinase-resistant penicillins, and those isolated in Poland came from a hospital where the penicillinase-resistant penicillins have never been used.

In spite of the diversity of source all the recorded strains show a similar, rather unusual, type of resistance. In addition all show high penicillinase-producing activity, and multiple resistance to other antibiotics and nearly all belong to one of only a few bacteriophage types of Group III. In all cases strains resistant to methicillin show cross resistance with all the other penicillinase-resistant penicillins.

Type of Resistance

Methicillin-resistant staphylococci isolated *in vitro* and in clinical practice differ in the type of resistance, although in neither case do the strains show an increased capacity to inactive methicillin. *In vitro*, when methicillin-sensitive strains are passaged in medium containing methicillin they give rise to cultures which show uniform increased tolerance to the antibiotic. With penicillinase-producing organisms the resistant mutants in other respects resemble the parent cultures and the resistance is usually stable for many generations in the absence of antibiotic. With penicillin-sensitive strains, however, the resistant mutants tend to be poorly growing and relatively avirulent resembling cultures of *Staph. aureus* rendered tolerant to benzyl penicillin *in vitro* (Barber, 1961).

On the other hand, naturally occurring methicillin-resistant strains, when tested in the usual laboratory media, grow quite abnormally in the presence of methicillin, and show a gross difference in sensitivity according to the